





Steven M. Ruben
Appl. No. 10/662,429

Department	
Subject	
Name	<u>Ann Kim #3</u>
Address	<u>8/23/93 → 10/27/93</u>
	43-648
Computation Notebook	
Dennison Stationery Products Co., Framingham, MA 01701	
	75 Sheets 11 1/2" x 9 1/2" 4x4 Quad.
0 73333 43648 8	

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Ruben EXHIBIT #87

Department _____
Subject _____
Name Ann Kim #3
Address 8/22/73 → 10/27/73
 43-648
Computation Notebook
Dartmouth Stationery Products Co., Framingham, MA 01701

0 73333 43648 8
75 Sheets
11" x 9"
4x4 Quad.

Ruben EXHIBIT 2087
Ruben v. Wiley et al.
Interference No. 105,077
RX 2087

New clones

48

Tuesday 9/7/93

Inoculate 5ml ~~4B~~ TB with 2 colonies
of:

HTPBC 29	A+B
H1B CW 23	A+B
HTPAN 08	A+B

for Boiling mini preps. —

Also inoculate DMase p A4 + A5 for
Boiling minis

Wednesday 9/8/93

use 1ul of each sample in a PCR Run

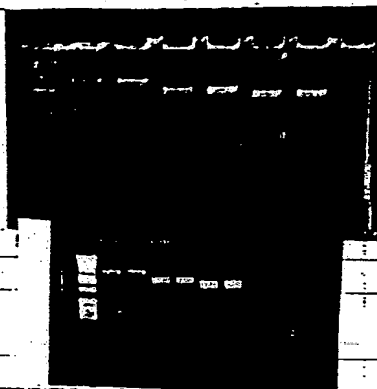
		6x	Run Program #69
M13F	0.15	0.9	95°C 5min
M13R	0.15	0.9	95°C 20sec
Tag	0.15	0.9	65°C 20sec } 30x
10x dUTP	1.6	9.6	72°C 1min
10x T Buffer	3.2	19.2	72°C 7.5min
H ₂ O	25.95	154.5	
ON	1		
	32ul		

Run 3.5ul on gel. —

Clean up PCR preps —

Add 30ul 10% PEG 1000 / 1.6M NaCl
Sit on ice 15min
Spin 3.3K 40min
Remove supernatant
3x 70% Ethanol. (150ul)
Spin 10min

Before



After

Dry
Resuspend in 30ul
Run 3.5ul on gel

48

9/8/93

Wednesday

Boiling Mini prep

HTPBC 24 A+B

HIBCW 23 A+B

HTPAN 08 A+B

DMSO P 44 A5

Spin 1.5ml of ON Culture

Remove supernatant

Resuspend pellet in 700ul STE

Boil 1 min

Spin 10 min

Remove pellet

Add 600ul 13% PEG / 1.6M NaCl

Vortex

Spin 15 min

Remove supernatant

1x 70% Ethanol Wash 1.3 ml

Vortex

Spin 5 min

Remove supernatant

Let air Dry

Add 100ul H₂O

Rem 5ul on gel

HTPBC 24 A+B

HIBCW 23 A+B

HTPAN 08 A+B

DMSO P 44 A5

Have sequenced - Clone Verification

forward & Reverse

Mama Glycobi

HTPBC 24 See
HTPAN 08 pg 72

New Clones

45

Thursday

9/9/93

Inoculate 5ml TB+amp. R4.
Wdr - HE8A429 A+B

HIBEC 69 A+B (THROMBON)

Inoculate 5ml LB+amp. Wdr

HTPBC24 A+B

HTPAN 08 A+B

HIBCW A+B

for Glycerol Stocks.

Boiling Mine Pups.

HE8A429 A+B

HIBEC 69 A+B.

9/10/93

Spin 1.5ml of ON culture.

Remove Supernatant.

Resuspend pellet in 600ul STE

Boil 40 sec.

Spin 5 min.

Remove pellet

Add 600ul 8 PEG/NaCl.

Vortex

Spin 15 min.

Remove Supernatant

Wash 1x with B0 1000ul 70% EtOH (dnd)

Vortex

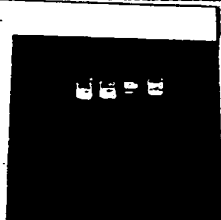
Spin 5 min.

Remove Supernatant.

Let air Dry

Resuspend in 50ul H₂O

Run 1ul on gel.



Dilute to 15 ul to 30 ul

and submit.

for Sequencing

Forward & Reverse

"Clone Verification"

HIBEC 69.

HE8A429 - 8cl pg

72

HIBEC 69 - 8cl

pg 71

72 New Clones
9/20/93 Monday

(pg 44a/b)

Inoculate 100 ml LB + Amp + Kan w/ the

HIBEC 6	- Thrombin
HIBCW 23	- Serum Inducible Keratin
HEAY 29	- IL-2
HTPAN08	- Tumor Necrosis Factor α
HTPBC 24	- Leucocyte Elastase Inhibitor

Incubate 37°C w/ aeration overnight

9/21/93 Tuesday

Cultures did not grow -
the vector does not contain
Kan Resistance -

Re inoculate into TB + Amp.
forgot to inoculate HTPBC 24
inoculate DNase P 44

9/23/93

Wednesday

Inoculate 100 ml TB + Amp with HTPBC 24 & R.

Dragon Midi Prep

Pour into 50 ml Conical
Spin 3.5K 10min
Remove Supernatant
Resuspend pellet in TE/Nall and combine
the 2 tubes of each into 1 tube
Spin 3.5K 10min
Remove Supernatant
Resuspend pellet in 4ml P1 Buffer + RNase
Making sure there are no clumps
Add 4ml Buffer P2 + mix gently
Incubate 5min at RT

(pg 77)

New Clones

77

pg 72

Wednesday

9/22/93

Diagen Media Prep Cont.

Add 4ml Chilled P3 Buffer + mix gently.

Incubate on ice 15 min

Spin 11K in GSA for 30 min 4°C

Get Column Ready by adding 4ml QBT Buffer

Let Column Drain by gravity.

Add Supernatant of Spin to column

Filter through Kimwipe

Let flow through

Wash Column with 2x 10ml QC Buffer

Let Flow through

Collect Flow Eluted Plasmid in 50ml

Conical - use 5ml QF

Transfer Eluted Sample to 30ml Corx tubes

Add 3.5ml (0.7 volumes) of isopropanol

Vortex

Spin SS-4 11K for 1 1/2 hrs.

Pour off supernatant

Wash in 70% Ethanol 14mls

Spin 11K for 10 min

Pour off Supernatant

Allow to dry 5-10 min

Resuspend pellet in ddH₂O

Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm 280.0 nm	280.0 nm 260.0 nm
1 DMSO P	0.0202	0.0125	0.0029	1.8177	0.5502
2 HTPANOB	0.0269	0.0152	0.0010	1.8253	0.5478
3 HIBCW23	0.0345	0.0185	-0.0021	1.7789	0.5621
4 HEBAN29	0.0273	0.0156	0.0025	1.8884	0.5295
5 HIBEC69	0.0268	0.0138	-0.0023	1.8074	0.5533

Run 5ul on a 1% gel with BlueScript II



78

~~DATA~~ New Clones

9/22/93

Wednesday

Have Sequenced Forward & Reverse

HTPAN08 FOR

DNase P FOR

H1BCW 28 FOR

HE8A129 FOR

H1BCW129 FOR

} 250 ng/ul in 10 ul
- gave ~30 ulSequence - Dig Terminator by DNA + 3.2 pmol Primer
H1BCW129 with Thymine 680-5

HE8A129 with HE8A129 202-5

HTPAN08 with HTPAN08 157-5

84

New Clones

9/22/93

Wednesday

pg 71

HIPANOR 157-S - 26mer

Deprotected 50°C overnight
 Dried down 250 μ l & resuspended
 in 100 μ l dd H₂O.

Dilute 1:250. Read OD_{260/280}

abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm 280.0 nm	280.0 nm 260.0 nm
0.1479	0.0885	-0.0004	1.6688	0.5992

1.22 μ g/ μ l

$$(26 \times 330) = 8580$$

$$1.22 \mu\text{g}/\mu\text{l} / 8580 = 142.2 \text{ pmol}/\mu\text{l}$$

Dilute 4.5 μ l primer into 195.5 μ l H₂O to
 get 3.3 pmol/ μ l.

Store -20°C

Na Clones

143

10/21/93

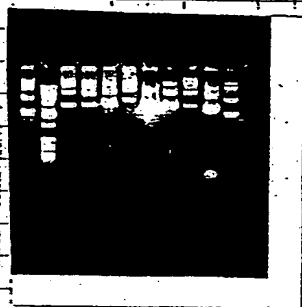
Sample ID	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm 280.0 nm	280.0 nm 260.0 nm		
21mer1710-HA5437	0.0620	0.0337	0.0006	1.8510	0.5402	0.51 ug/l	726 pmol/l
22mer2711-HA58463	0.0728	0.0447	-0.0000	1.6298	0.6136	0.6 ug/l	826 pmol/l
22mer3712-HPRAT312	0.0471	0.0220	-0.0004	2.1219	0.4713	0.39 ug/l	53.7 pmol/l
21mer4713-HA 4585	0.0536	0.0303	-0.0006	1.7530	0.5705	0.44 ug/l	63.5 pmol/l
20mer5714-HA 7642	0.0355	0.0199	-0.0009	1.7513	0.5710	0.29 ug/l	43.9 pmol/l
20mer6715-HA 7645	0.0378	0.0219	-0.0001	1.7237	0.5802	0.31 ug/l	47 pmol/l

Dry down Sequencing Oligos for Eggs
Carice

Monday

10/25/93

Run Plasmids on gel with PBSK & 1kb ladder



11 PBSK
2 Marten
3 DNAse P
4 HTPAN08
5 HIBCW23
6 HESAH29
7 HTPBC24
8 HIBECW109
9 HTBAS35
10 HMP8479

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